REMARKS

The Office Action dated September 26, 2003 presents the examination of claims 1-14. Claims 2-6 and 13 are amended. No new matter is inserted into the application.

Request for Interview

If, for any reason, the instant Reply does not immediately place the present application into condition for allowance, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. 45,702) at 703-205-8000 to schedule a personal interview at the Examiner's convenience, prior to the issuance of an Advisory Action.

Rejection under Obviousness-type Double Patenting

The Examiner maintains the rejection claims 1-14 under the judicially created obviousness-type double patenting rejection over claims 1-21 of U.S. Patent 6,372,960. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

In order to overcome this rejection, Applicants submit herewith a Terminal Disclaimer in compliance with 37 C.F.R. § 1.321(c). The Terminal Disclaimer is signed by a registered attorney of record in the present application in compliance with 37 C.F.R. § 3.73(b).

Applicants respectfully submit that the filing of the Terminal Disclaimer overcomes the obviousness-type double patenting rejection. Withdrawal thereof is therefore respectfully submitted.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner rejects claims 2-6 under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Specifically, the Examiner points out that the recitation of "said barstar DNA" in claims 2-6 lacks antecedent basis in newly amended claim 1. In order to answer this rejection, claims 2-6 are amended to recite barstar "coding sequence" as supported in claim 1. Thus, the instant rejection is overcome. Although not specifically mentioned by the Examiner, claim 13 is also amended accordingly.

Rejection under 35 U.S.C. § 103

The Examiner maintains the rejection of claims 1-2 and 7-14 for allegedly being obvious over EP 412,911 in view of Koziel et al. (1993) and Hartley (1993). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner asserts that it would have been obvious to one of ordinary skill in the art to modify the GC content of the native barstar coding sequence for improved expression in monocots. However, the Examiner acquiesces that there was no motivation to modify the barstar coding sequence for improved expression in dicots, and implies to claims directed to a process for the expression of barstar in dicot plants only would be allowable.

Although Applicants acknowledge the patentability of claims directed to dicots, Applicants respectfully submit that the rejection is still improper because there was no motivation to modify the native barstar gene in either monocot or dicot plants, for the fact that the native barstar gene of EP 412,911 was well expressed in monocot as well as in dicot plants. Further, the problems with the expression of the *Bacillus thuringiensis* δ-endotoxin gene in plants in general and the solution reported by Koziel *et al.* (and Perlak *et al.*) cannot just be extrapolated to the native barstar gene for which there were in fact no expression problems. Thus, the Examiner's relies on Koziel *et al.* to teach a solution for which there was no problem. The Examiner maintains that it is well known in the art that corn and other monocots have a higher requirement for GC usage than dicots. However, the Examiner is reminded that the level of skill in the art cannot be relied upon to provide the suggestion to combine references. Al-Site Corp. v. VSI Int'l Inc., 174 F.3d

1308 (Fed. Cir. 1999). Instead, the prior art itself must suggest the desirability of the claimed invention. Even if a combination of references teaches every element of the claimed invention, without a motivation to combine, a rejection based on a prima facie case of obvious is improper. In re Rouffett, 149 F.3d 1350 (Fed. Cir. 1998).

The present invention teaches a barstar coding sequence with an AT content of less than 40% (claim 1) and a barstar coding sequence with an AT content of less than 40% and a codon usage that is optimized for (*dicot plants such as*) oilseed rape, cotton, (*as well as monocot plants such as*) maize, rice and wheat (claim 2), which, when expressed in a plant cell, is capable of improved inhibition of barnase.

EP 412,911 teaches the native *Bacillus amyloliquifaciens* barstar gene with an AT content of about 51.6% which was found to be suitable for inhibition of barnase, in dicots such as oilseed rape and tobacco (*as recognized by the Examiner*), as well as in monocots such as rice and corn (*see following examples*). Examples of successful applications of the native barstar gene in various species can be found in *e.g.*:

- WO 01/41558 (US 6,506,963) that describes the commercially used barnase/barstar system for winter oilseed rape (*see also previous response*);

- WO 92/13956 (US 5,639,948) that teaches rice (Example 7), corn (Example 8) and tobacco (Example 9) expressing the native barstar gene under the control of stamen-specific promoters from rice; and

- WO 96/26283 (US 6,025,546 and US 6,344,602) that teaches rice (Example 1), corn (Example 2) and oilseed rape (Example 3) expressing the native barstar gene under the control of the constitutive 35S promoter.

Koziel *et al.* teaches a synthetic version of the *Bacillus thuringiensis* δ-endotoxin gene with an AT content of 35% which produced significantly higher amounts of the encoded protein in maize than the native *Bacillus thuringiensis* δ-endotoxin gene with an AT content of 62% which did not produce detectable levels of protein in maize.

Koziel *et al.* state themselves on page 194, right column, lines 6-9: "Our attempts to express detectable levels of CryIA(b) protein in maize using a truncated version of the native coding sequence have not been successful; therefore a modified coding sequence was used." Thus, the motivation to modify the coding sequence of the native *Bacillus thuringiensis* δ-endotoxin gene for expression in maize was that this construct was found not to be well expressed in maize.

In contrast, there was no motivation to modify the coding sequence of the native barstar gene for expression in any plant,

since it was well expressed in both monocot and dicot plants (*see Examples above*). Again, the Examiner cannot merely use hindsight to apply the teachings of Koziel *et al.* to the native barstar gene when one of ordinary skill in the art would not be motivated to do so. The fact that references may be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. In re Mills, 916 F.2d 680 (Fed. Cir. 1990).

Koziel *et al.* increased the GC content of the native *Bacillus thuringiensis* δ-endotoxin gene in an attempt to enhance its expression in maize, in view of the teachings of Murray *et al.* (1989, *Nucl Acids Res* 17:477-498) and Perlak *et al.* (1991, *Proc Nat Acad Sci USA* 88, p.3324-3328). Indeed, Koziel *et al.* state on page 194, right column, lines 1-6: "Plants in general have a higher GC content than that found in the δ-endotoxins, with maize having an even more pronounced preference for high GC content in coding regions (Murray *et al.*). Modifying the coding sequence to increase the GC content of the native gene results in a dramatic increase in expression of the insecticidal protein (Perlak *et al*)." Perlak *et al.* also state themselves on page 3324, left column, last paragraph, line 6: "Poor expression in plants is a well-reported characteristic of the *Bacillus thuringiensis* var. *kurstaki* insect control proteins".

Thus, again, the motivation to modify the coding sequence of the native *Bacillus thuringiensis* δ-endotoxin gene for expression

in plants was that it was not well expressed in plants. By contrast, there was no motivation to modify the coding sequence of the native barstar gene for expression in any plant, since it was well expressed in both monocot and dicot plants.

Perlak *et al.* goes on (page 3324, right column, line 5) to say, "Examination of the insect control protein gene coding sequence indicated that it differs significantly from plant genes in G+C content. Multiple sequence motifs that are not common in the coding region of plant genes were found to be common in the wild-type *cryIA(b)* sequence. These included localized regions of A+T richness resembling plant introns, potential polyadenylation signal sequences, ATTTA sequences, which have been shown to destabilize mRNA in other systems, and codons rarely used in plants."

For example, Perlak *et al.* found that one region (the region of oligonucleotide B in Fig. 1) in the CryIA(b) gene was critical to increased expression. This region of oligonucleotide B "contains three potential polyadenylation signal sequences (two AACCAA and one AATTAA)", leading Perlak to suggest, "it is possible that the incomplete functioning of a polyadenylation signal led to processing without polyadenylation causing instability. Another possibility is that the region of oligonucleotide B contains an as yet undefined regulatory sequence dependent on the context of its surrounding sequence." (See, page 3328, left column, second paragraph).

These characteristics provided a motivation for Perlak to construct a partially (called PM) and a fully modified (called FM) version of the *Bacillus thuringiensis* δ-endotoxin gene with an AT content of 59% and 51%, respectively, which, respectively, produced up to 10- and 100-fold higher amounts of the encoded protein in tomato than the native *Bacillus thuringiensis* δ-endotoxin gene with an AT content of 63%.

By contrast, as argued before, the native *Bacillus amyloliquifaciens* barstar coding sequence (SEQ ID NO:1) does not differ significantly from plant genes in G+C content and no one in the cited art has identified specific regions of A+T richness that are critical to its expression. Consequently, there was no reasonable expectation of success that decreasing the AT content of the native *Bacillus amyloliquifaciens* barstar coding sequence from 51.6% to less than 40% would have any effect on the expression of barstar and if so, that this would result in an improved inhibition of barnase.

It is not denied that monocot plants, like maize, have been described by Murray *et al.* to have a preference for high GC content in coding regions. See Murray *et al.*, page 479: "In general, the most important factor in discriminating between monocot and dicot patterns of codon usage is the percentage G+C content of the degenerate third base. In monocots, 16 of 18 amino acids favor G+C in this position, while dicots only favor G+C in 7 of 18 amino acids." However, Murray *et al.* also state "To our

knowledge, no one has experimentally altered codon bias in highly expressed plant genes to determine possible effects of the protein translation in plants to check the effects on the level of expression (page 491, last paragraph, lines 6-8)."

Hence, there was no reasonable expectation of success that the adaptation of the coding bias of a transgene which is well expressed in dicot as well as in monocot host plants to the codon bias of those hosts (*e.g.* by adapting the codon usage of the native barstar to dicot plants, like oilseed rape and cotton, as well as monocot plants, like maize, rice and wheat as described at least on page 12, line 31, to page 13, line 21 and on pages 21-22 of the instant application) would further improve its expression in both dicot and monocot host plants.

Indeed, it is not obvious that the adaptation of the coding usage of a transgene to be more similar to that of the host will improve the expression of any transgene even if it is already well expressed in that host. For example, Fiers *et al.* teaches that modification of the codon usage of the eukaryotic hIL-2 gene to be more similar to that of *E. coli* did not further improve the expression level of the eukaryotic hIL-2 gene in *E. coli*, which was already high (*Proceedings of the 8th International Biotechnology Symposium*, Paris, 1988; p. 690, lines 3-12; a copy of which is attached hereto). They also emphasize, "[T]here are numerous examples of heterologous genes containing "unsuitable"

codons and which nevertheless are expressed at high yield in *E. coli*." (See, page 690, lines 13-16).

In this respect, it should also be noted that although Hartley *et al.* (1993) teaches that the barstar protein can be modified by substituting a variety of differently-charged amino acids at a variety of residues without affecting protein function (as stated by the Examiner in paper 6, page 5, lines 4-6), "their yield varied from a few hundred microgram equivalents per liter to the 100-500 mg/L produced by the wild-type" (see, page 5981, left column, last paragraph, lines 3-6; see also page 5981, right column, lines 6-8) and at least three mutants have been described that have no detectable barstar activity (see page 5981, left column, last paragraph; lines 6-9).

In conclusion, Applicants strongly disagree with the Examiner's assertion that the skilled artisan would be motivated to modify the GC content of any transgene for expression in monocots, given that there was:

- **no motivation**:
  - the native *Bacillus amyloliquifaciens* barstar coding sequence (SEQ ID NO:1) was well expressed in monocot as well as dicot plants (*e.g.* EP 412,911; US 6,506,963; US 5,639,948; US 6,025,546 and US 6,344,602).
- **no reasonable expectation of success**:

- In contrast to the native *Bacillus thuringiensis* δ-endotoxin gene, the native *Bacillus amyloliquifaciens* barstar coding sequence does not differ significantly from plant genes in G+C content and is not known to have specific regions of A+T richness that are critical to its expression.
- Although monocot plants, like maize, were described by Murray *et al.* to have a preference for high GC content in coding regions, it is not obvious that altering codon usage in a transgene which is well expressed by adapting the codon usage of the gene to a series of plant species, dicot as well as monocot plants, further improves its expression in both dicot and monocot host plants (see the teachings of Murray *et al.*, Fiers *et al.* and Hartley *et al.*).

For all of the above reasons, the instant rejection is clearly improper and must be withdrawn.

Conclusion

Applicants respectfully submit that the above amendments and/or remarks fully address and overcome the rejections and objections of record. The instant claims are now in condition for allowance. The Examiner is respectfully requested to issue a Notice of Allowance indicating that claims 1-14 are allowed.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), the Applicants hereby petition for an extension of one (1) month

to January 26, 2004 in which to file a reply to the Office Action. The required fee of $110.00 is enclosed herewith.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By Kristi L. Rupert #45,702
for Gerald M. Murphy, Jr., #28,977

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

GMM/KLR
2428-0108P

Attachments:
(1) Terminal Disclaimer
(2) Vers Fiers *et al.* (1988) Engineering maximal expression of heterologous genes in gram-negative bacteria. *In:* Proceedings of the 8th International Biotechnology Symposium (Paris, 1988), Volume II, p. 680-694.